Preparation of samples

Receipt and storage: There are important notes about the food sample include:

1- **Size of sample for examination**: The quantity of sample submitted should normally be at least 100g.

2- **Handling for examination**: Contamination of the sample and microbial growth or death during sampling transport and storage should be avoided. Aseptic handling techniques should be used throughout the sampling process.

3- **Containers:** Sampling instruments and containers that come into direct contact with food should be sterile. Samples taken from unpacked or opened cans or packets of food should first be placed in clean, dry, and sterile leak-proof containers such as wide mouth glass of plastic jars with closures. The contained sample should be secured with a tamper evident seal and labeled. Information recorded on the label should include the name of the food, names of the sampling officer the place, date and time of sampling and a unique tagging identification number. If the label is likely to become damaged during transport the sample, so should be placed in a second container, such as a plastic bag and sealed to prevent tampering.

4- **Transportation and storage**: Samples should be transported and stored under conditions that inhibit changes in microbial numbers, so there are different ways to protect the samples until examination:

- Frozen foods need to be kept frozen as far as possible.
- Chilled/refrigerated foods and other perishable foods need to be kept in a surrounding air temperature at or below 8C^{o} and preferably between 0C^{o} and 4C^{o} but not frozen.
- Hot or warm samples should be kept separate from other food samples and cooled down as quickly as possible to a temperature of 8 C or below.

- Dried foods, un-blown cans and other shelf-stable items need not be cooled but should be stored and transported at a temperature less than 40 C^{o}.

Refrigerated insulated containers or insulated containers cooled by means of ice or gel packs should be used to hold and transport chilled or frozen samples. If frozen packs are used their volume should form at least 10% of the volume of the insulated container.

Samples should be delivered to the laboratory as soon as possible, preferably within 4h. If there is likely to be a delay the samples must be stored under conditions that will minimize microbial change.

5- Request for examination: All relevant information should accompany a food sample to ensure that it is subjected to the most appropriate examination and to enable the food examiner to interpret the results:

- Name and authority of sampling officer.
- Sample identification number.
- Date, time, and place of sampling.
- The temperature and storage conditions at the place of sampling.
- Description of sample including batch/lot number, canning code, etc. and durability date (use by, best before, etc.)
- Name of owner, manufacturer, importer, seller, buyer, as appropriate.
- The process and date of cooking (if known) of cooked foods.
- Country of origin
- Other relevant storage factors, e.g., condition of packages, humidity, sanitation.
- Method of sampling (random throughout lot, random throughout accessible units, otherwise).
- Clinical and epidemiological details (in cases of suspected food poisoning).
- Storage and transport conditions.
- The air temperature of the cool box should be recorded on arrival in the laboratory.

6- Receipt and description at the laboratory: The following details should be recorded on the report form:

- **Type of packaging** this may influence the condition of the contents and should be recorded to aid interpretation of the results. For example, the environment within vacuum packages is anaerobic. The gas mixtures used for modified atmosphere packaging will also influence the microbiology of a food. defects such as dents, and imperfect seals should also be noted.
- **Appearance** describes the food sample in general terms, e.g.70g of machine-sliced, paper-wrapped, pink-colored, signs of deterioration, abnormal color and mold should also be recorded.
- **Texture** bacterial deterioration can cause products to become soft or semiliquid; this applies particularly to meat products.

Methods for Microbiological Examination of Foods

The aim of microbiological examination of foods is to identify the presence, types, and numbers of microorganisms and/ or their products (such as toxins) in food samples, which cause the spoilage of the food or pathogenic infections for the consumers.
In general, there are four different microorganisms we are looking for:

1. **Indicator organism/** Coliform group and *E. coli, E. coli* indicate the presence of pathogenic enterobacteriacea.
2. **Food poisoning organisms:**
    - Those which can grow in food cause disease by infection
    - Those which produce toxins in food cause intoxication
3. **Infectious organisms:** Food act as a vector not as a growth medium
4. **Spoilage organism:** this mostly include fungi (yeast and molds)

Dilution
Diluent 0.85%NaCl, 0.1% peptone, Phosphate buffer saline (PBS)

Microbiological Examination Methods

There are many different methods: -

1- Microscopic examination of microorganism.
2- ATP photometry (ATP bioluminescence)
3- Plate count
4- Breed smears (milk)
5- Flow cytometry
6- Dna hybridization
7- Polymerase chain reaction (PCR)
8- Membrane filtration- direct epiflourescent technique (DEFT).
9- Most Probable Number Method (MPN)

Microscopic examination of microorganism

In this method there is a specific slide that can be used for this purpose which is: -

- Small sample volume & rapid technique
- Inexpensive equipment
- Total cell (living & dead cells)
- The examination of Liquid & Semi-solid Foods

ATP photometry (ATP bioluminescence)

It is a Rapid method (1-2 min) which break down the non-microbial cells in food Remove non-microbial ATP using ATPase Release ATP from bacteria cell Addition of luciferin & luciferase Record light emission (ATP photometry).

- This test Based on detection of ATP in metabolically active cells through the production of light.
- The amount of ATP per cell is generally constant.
- Release fluorescence light depend on the amount of ATP in the food sample.
- And ATP measurement based on bioluminescence using luciferin-luciferase (substrate-enzyme) complex relies on oxidation of luciferin by enzyme luciferase.
- Luciferin + luciferase + ATP + O2 ⟶ Oxyluciferin + luciferase + AMP + light
- The produced light, usually detected and quantified by sensitive luminometer device

Application

Starter culture, Test UHT milk*(Ultra-high temperature processing (UHT), Microbial contamination of meat chicken and beef and Surface contamination. However, this method could include bacteria & yeast cell

Rapid Method

A. Immunological Methods Enzyme-linked immunosorbent assay (ELISA)

Immunological methods rely on the specific binding of an antibody to an antigen. Immunoassay refers to the qualitative and quantitative determination of antigen and antibody in a specimen.

B. DNA/RNA Methodology

DNA hybridization: The identification of bacteria by DNA probe hybridization is based on the presence or absence of particular genes.

C. Polymerase Chain Reaction

The PCR is an in-vitro method used to increase number of specific DNA sequence in a sample. PCR is used increasingly in research in food microbiology because of its high sensibility or specificity. Polymerization is performed with the oligonucleotide as primers for the enzymes and the target DNA as template

Membrane filtration- Direct Epiflourescent Technique (DEFT)

DEFT is a direct method used for enumeration of microbes based on binding properties of flurochrome acridine orange. The number of viable cells is determined based on the count of orange cells on the filter and can be performed in 10 min.

Most Probable Number Method (MPN)

- Statistic approach to quantitate the numbers of bacteria, which utilize a multiple dilution to estimate the population of microorganisms in foods
- Use to estimate the number of M.O (less accurate than the plate)
- Examine large amount of sample
- The growth of M.O in the medium appear as turbidity/change in color of medium
- Time consuming

Note

A major disadvantage of alternative and rapid methods over cultural methods is that most methods need damaging of the cells and therefore, viable cells for confirmation and further characterization can only be obtained by repeat analysis using standard cultural procedures. Moreover, rapid methods usually detect only one specific pathogen, while cultural methods may simultaneously detect and isolate many pathogens by including several types of numerous microbiological examinations or samples, selective media in the analysis. The use of several rapid assays to do multi pathogen analyses on a food makes this analysis unacceptably expensive.

Indirect Methods:

Plate count

A- Pour plate B- Spread plate C- Drop plate

Culturing Technique

There are several different kinds of medium used:

1. General media (Nutrient agar NA for bacteria and potato dextrose agar PDA for fungi)
2. Selective media
3. Differential media
4. Diagnostic media

Results:

After 24-48 hours, count all the colonies (again**:** note that the embedded colonies will be much smaller than those which happen to form on the surface). A magnifying colony counter can aid in counting small, embedded colonies.

Calculate CFU/mL using the formula: CFU/mL= CFU * dilution factor * 1/aliquot

(The volume of diluted specimen (aliquot) is either 0.1 or 1.0 mL)

Disadvantages of Pour plate method

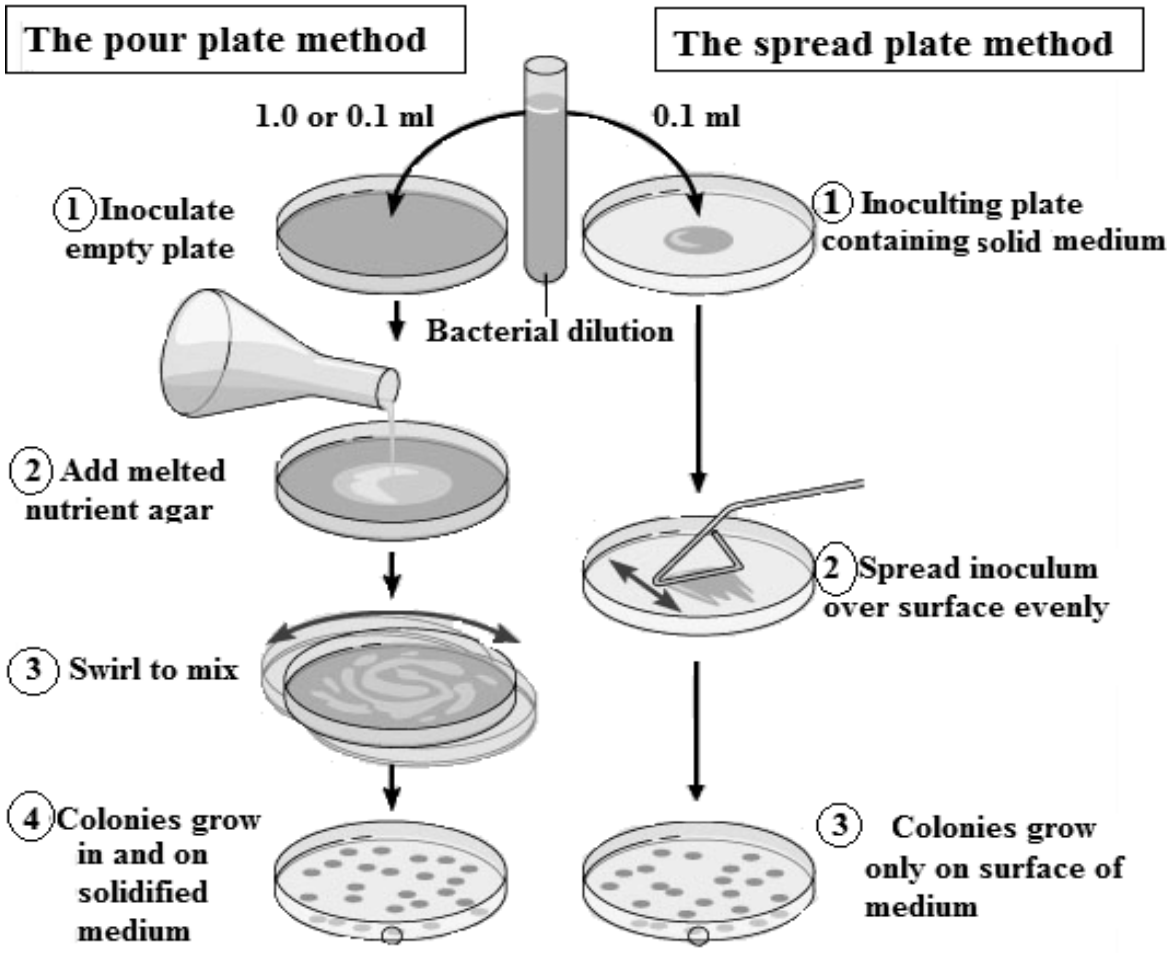
1. Preparation is time consuming compared with streak plate/and or spread plate technique.
2. Loss of viability of heat-sensitive organisms coming into contact with hot agar.
3. Embedded colonies are much smaller than those which happen to be on the surface. Thus, one must be careful to score these so that none are overlooked.
4. Reduced growth rate of obligate aerobes in the depth of the agar.

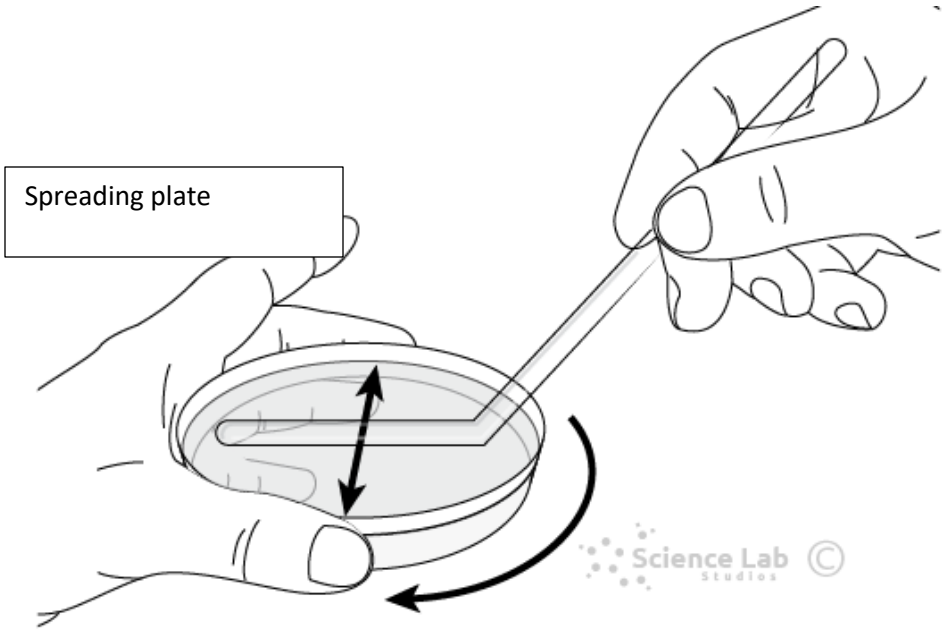
Procedure of culturing technique

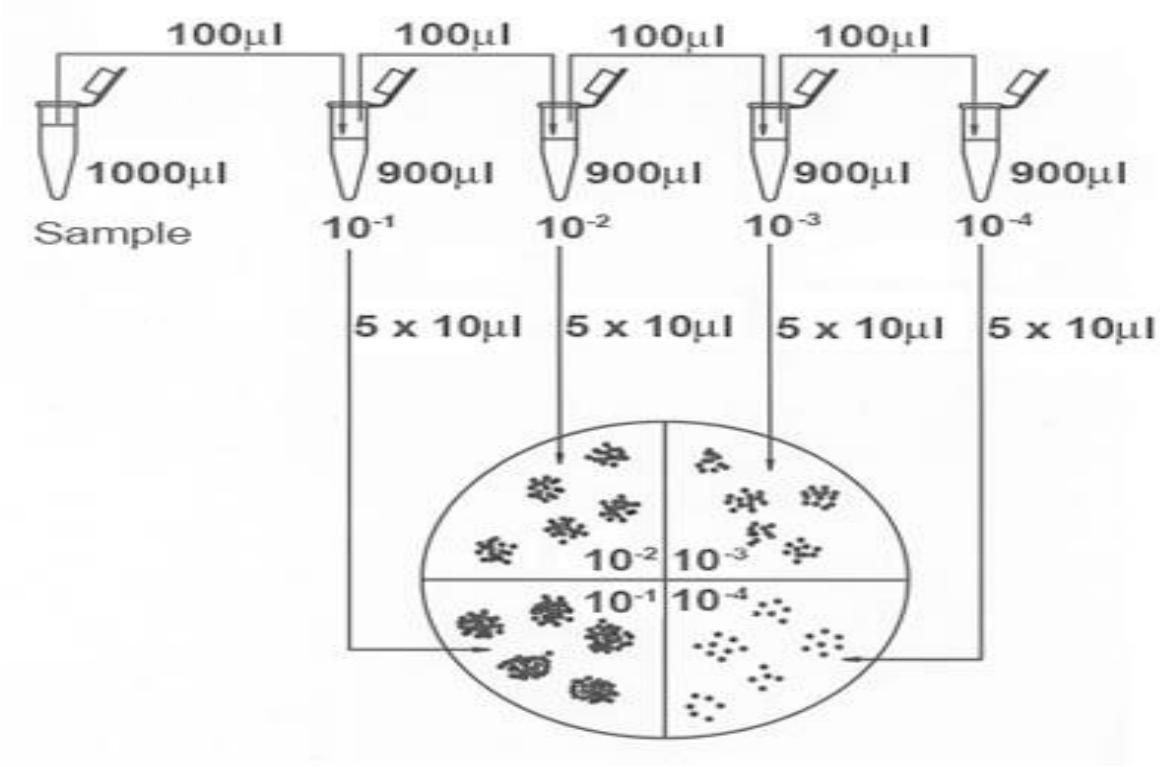
1. Prepare the dilution of the test sample expected to contain between 30-300 CFU/ml. *(Follow serial dilution technique)*
   - Inoculate labeled empty petri dish with specified mL (0.1 or 1.0 mL) of diluted specimen for pouring plate method. Then Pouring the molten agar on the petri that has specimen.
2. Collect one bottle of sterile molten agar *(containing 15 mL of melted Plate Count Agar or any other standard culture media)* from the water bath (45°C).
3. Pouring the molten agar medium in an empty petri dish in the spreading and dropping methods.
   a) Hold the bottle in the right hand; remove the cap with the little finger of the left hand.
   b) Flame the neck of the bottle. Then Lift the lid of the Petri dish slightly with the left hand and pour the sterile molten agar into the Petri dish and replace the lid.
4. Flame the neck of the bottle and replace the cap.

5. Gently rotate the dish to mix the culture and the medium thoroughly and to ensure that the medium covers the plate evenly. Do not slip the agar over the edge of the petri dish.
6. Allow the agar to completely gel without disturbing it, it will take approximately 10 minutes.
7. Seal and incubate the plate in an inverted position at 37°C for 24-48 hours.

Overview of Pour plate method and spread plate method







Dropping plate method

Microbiological Examination of Milk

The milk is an excellent medium for growth bacteria, yeasts, and molds. The microorganisms are important in milk and dairy products for three principal reasons:
1. Pathogens or their toxins may constitute health hazards.
2. Spoilage microorganisms or their metabolites may cause spoilage.
3. Lactic acid bacteria and others may contribute to the preservation of milk and in the production of desirable flavor and physical characteristics.

****Three sources** contribute to the M.O. in milk: udder (interior), teat (exterior), milking and equipment.

****There are** five major parameters are routinely checked by regulatory agencies for quality raw milk production:
1. Mastitis diagnosis through somatic cell counts and microbiological analyses.
2. Determination of microbial counts in bulk tank milk for verification of hygiene (cleaning, disinfection, cooling).
3. Testing for veterinary drugs and aflatoxins.
4. Spoilage/ Determination of total viable counts
5. Determination of psychrotrophic or thermotropic microorganisms.

Milk Examination:

- Microbiological tests are applied to detect poor hygiene conditions and to evaluate the microbial quality of raw and processed milk.
- Total bacterial load is used to get an overview of the microbial contamination.
- Psychrotrophic microorganisms are assessed to prevent potential spoilage.
- Coliforms are assessed to evaluate the hygiene history of milk.
- Specific pathogens (e.g. *Salmonella* spp., *S. aureus*, *Listeria monocytogenes*, *Mycobacterium paratuberculosis*) to prevent potential health hazards.
- Thermoduric bacteria to assess the hygiene of the production system.

Screening the quality of milk

1. Dye- reduction test.

a. Methylene blue Reduction test (Reductase test, Reduction test):

This is a rapid test to find relative number of bacteria in a milk sample. The length of time for color change in a specific dye is **proportional** to the number of bacteria in the sample (more bacteria present, lead to faster reduction). On the other hand, the reductase test is based on the oxidation- reduction (O/R) activities of the viable bacteria present in milk.

b. Resazurin test:

Resazurin undergoes a series of color changes from normal slate blue to pink to colorless form depending on O/R potential.

Milk Quality	**pH**	**Color**	**Reading the results: Dye**
Acceptable	6.6	Blue (violet)	Resazurin
Moderate	5.3	Pink	Resarufin
poor	4.8	Colorless	Dihydroresorufin

2. Phosphatase test:

The phosphatase enzyme which is normally present in raw milk is destroyed by pasteurization and a test for residual phosphatase activity should be used to check that effective heat treatment has been achieved. The heat tolerance of this enzyme is similar to *Coxiella burnetti* (Q- fever organism) and *Mycobacterium tuberculosis* (tuberculosis bacterium).

The microbiological tests of milk

Standard Plate Count

The Standard Plate Count (SPC) of raw milk gives an indication of the total number of aerobic bacteria present in the milk at the time of pickup. Milk samples are plated in a semi-solid nutrient media and then incubated for 48 hrs. at 32°C to encourage bacterial growth. Single bacteria (or clusters) grow to become visible colonies that are then counted. All plate counts are expressed as the number of colony forming units per milliliter (cfu/ml) of milk. Newer films-based tests have allowed for automation of this procedure. The nutrient agar system remains the gold standard.

Coliform Count

The Coliform Count (Coli Count) procedure selects for bacteria that are most commonly associated with manure or environmental contamination. Milk samples are plated on a selective nutrient media that encourages the growth of coliform bacteria, while preventing the growth of others. Although coliforms are often used as indicators of fecal contamination, there are strains that commonly exist in the environment. Coliforms may enter the milk supply as a consequence of milking dirty cows or the claw becoming soiled with manure during milking. Generally, counts greater than 100 cfu/ml would indicate poor milking hygiene or other sources of contamination. Higher coliform counts more often result from dirty equipment and in rare cases result from milking cows with environmental coliform mastitis.

The Breed count:

1. Amount of 0.01 ml of the sample spread over 1cm of a microscopic slide then the film is dried. Add some drops of xylene to remove the fat (for 1 min). Fix by steam in the boiling water bath (5 min).
2. Then wash the slide with alcohol to remove the xylene ----- wash with D.W to remove the alcohol. After that stain the slide with M.B. (methylene blue, 15 sec). Then scanned under microscope ---- Enumerate.

Biochemical tests used to characterize bacteria

A. **Catalase Test:**

The catalase test determines if an organism can degrade peroxides (i.e.) to oxygen and water. The enzyme catalase is present in certain bacteria as a protective feature to destroy toxic peroxides. When hydrogen peroxide is added to a culture of bacteria that has the catalase enzyme, visible bubbles of oxygen are liberated during its degradation. Most Gram-negative bacteria common in dairy products are catalase-positive. The catalase test is most useful in distinguishing between certain Gram-positive bacteria (catalase-positive *Micrococcus versus* catalase-negative *Streptococcus* or *Lactococcus*).

Procedure:

1. From an agar plate, transfer a small amount of a colony to the surface of a clean dry slide with a sterile loop or applicator stick. Alternatively, the test can be done directly on a colony.
2. Add one drop of 3 - 5 % Hydrogen Peroxide.

B. Liquid Reagent (oxidase reagent):

Procedure

1. Prepare the "oxidase" reagent just before use. Weigh 0.1 gram of para-aminodimethylaniline oxalate and dissolve in 10 ml of distilled water with gentle heating. This reagent is available prepared in sealed vials.
2. Soak an area of filter paper with the (oxidase reagent).
3. Using a wooden applicator stick, toothpick, or platinum loop (do not use standard loop material), apply a portion of bacterial growth from an isolated colony to the moistened area.
4. If the organism is **oxidase-positive** the reagent will turn the growth **red to black** within 2 minutes. If they are **oxidase-negative**, **no color** will develop.

Microbiological Examination of Meat and Fish

-The meat and fish are food rich with protein, lipids, vitamins, and minerals, as well as have a good water content (moisture) with a suitable pH number (near the neutral). These all factors are suitable for growing and reproduction most of microorganisms.

-The unblemished animal free from microorganism but during cutting the animal will have contamination from intestines, skin, foot, environment, from workers, tools, water, and others.

-Bacteria may be present in meat include:

- *Pseudomonas*
-*Bacillus*
-Lactic acid bacteria
-Coliforms.

-Fish faster than other meat in spoilage because it has high moisture (high water content which suitable for the enzymatic activity of most microorganisms.

- **Bacteria** mostly present in fish is belonging to the genus *Pseudomonas*.

-The source of bacteria in fish is the contaminated water addition intestines, gills of fish, or during cleaning the fish.

Method:

A) Estimation surface contamination of meat:

By swab, take a smear from surface of meat and put it in tube contain 5 ml of D.W. then mix well.

*Microscopic exam:

Prepare a slide from swab water and stain by gram stain exam of *Pseudomonas* and other bacteria as previously mentioned.

*Total number of bacteria:

1-Transfer loop full of swab water and streak on surface media like nutrient agar and *Pseudomonas* agar, as well as plate count agar;

2- Incubate the Petri dishes at 30-37C^{o} for 48hrs.

3- Compare growth on the three media.

4- Then total number of bacteria.

*Mold and yeasts:

1- Prepare potato dextrose agar medium (PDA), adjusted the PH to 4
2- Sterilized the medium in autoclave.
3-pour the medium in petri plates, left them to solidify.
4- Transfer 0.5 ml of swab water to the plates and spreading it using a spreader.
5- Incubate the Petri dish at 22-25C^{o} for 3-5 days and count the number of fungal cells

B-Enumeration the total number of M.O.:

Enumeration the total number of bacteria, mold, yeast, coliform and some kind of pathogenic bacteria to know the kind of contamination in meat.

-Weight 11 gm. of meat and put it in blender with 99 ml of dilution solution 0.1% peptone.

- Mix for 2 min

- Make serial dilutions until the dilution factor 10^{-5}.

-Then transfer of the suitable dilution and put in a Petri dish, add for it the suitable medium.

-Doing the following tests:

1-Total number of bacteria: Transfer 1ml of dilution to the Petri dish, add to its plate count agar or nutrient agar, incubate at 30-37 C for 2 days and count the number of bacteria in 11 gm.

2-Total Coliform: Transfer 1 ml of dilution to the Petri dish, add (VRB) violet red bile salt agar, incubate at 37C for 24h.Count the violet colony and estimate the number of Coliforms in 11gm.

3-Mold and yeast: Transfer 1 ml of dilution to the Petri dish, add to it PDA medium, incubate at 22°C for 5 days and count the number of fungi in 11 gm of meat.

Microbiological examination of Fruit and Vegetables

The microorganisms exist on the fruit and vegetables surface coming from soil, water, air, insects and others. This microorganism includes bacteria and mold that is capable of decomposition cellulose and pectin and inters into fruit and vegetables, then they will be activated and spoil this crop.

Vegetables are spoiled faster than fruit due to high pH in vegetables, while the low pH in fruit encourages the growth of molds and yeasts. The important vegetables's bacteria, which cause spoil are *Bacillus*, *Psedumonas* and *Erwinia*. While the molds are: *Alternaria*, *Penicillium*, *aspergillus* and *Fusarium* spoil fruits such as oranges and apples, whereas the fruit contain high amount of sugar such as grapes are spoiled by yeasts.

There is another group of food possesses characteristics allow some microorganism groups to grow, for example:

- Food with high percentage of sugars is suitable medium just for Osmophilic Microorganisms (*microorganisms* adapted to environments with high osmotic pressures). *Saccharomyces rouxii, Saccharomyces bailii, Debaryomyces and Saccharomyces cerevisiae*

- Salty food is suitable medium just for Halophilic Microorganisms. Haloanaerobium, Halobacteroides

- Dried food is suitable medium just for a few Microorganisms that can grow in low aqueous level, which called Xerophilic Microorganisms.

Microorganisms in dry fruit and vegetables

Dry fruit and vegetables contain variety of microbial groups. The source of contamination of some organisms from farm, which don't killed during the drying process because it is **spores forming** and **heat drying resistance**. Whereas other contamination comes from various sources such

as: after drying, through trading, during storage and during operations of re-hydration.

The pathogenic Microorganisms, which are revivals in dried fruit and vegetables don't threats health because they cannot grow and reproduce in low level of humidity. After the re-dehydration process of fruit and vegetables, some Microorganisms can grow and reproduce (May including pathogenic microorganisms). Therefore, it is important to perform quality and quantity microbial tests on dried food to control the food quality.

Methods:

1) Dry fruit: Place amount of 10 gm of dry fruit in a sterile beaker containing 90 ml of sterile D.W with gentle agitation. Leave the mixture for 30 minutes until rehydration.

A- Total bacterial number estimation: Amount of 1 ml and 0.1 ml of mixture transferred by pipette to duplicate Petri dishes and pour the culture media (nutrient agar). Then incubate them for 3 days (at 30 ^{o}C). Then, count the colony and estimate the total bacterial number for each gram.

B- Lactic acid bacteria: Amount of 1 ml of mixture transferred by pipette to duplicate Petri dishes and pour the culture media (orange serum and agar). Then incubate them for 2 days (at 30 ^{o}C). Then, examine the colonies by Catalase enzyme detecting.

C- Mold and yeast: Amount of 1 ml of mixture transferred by pipette to Petri dish and pours the culture media (Potato Dextrose Agar PDA). Then incubate them for 5 days (at 22 ^{o}C). Then, count the colony and estimate the total bacterial number for each gram. Then, count the colony and estimate the total mold and yeast number for each gram.

2) Dry vegetables: Samples are prepared as in the last case

A-Total bacterial number estimation: Prepared and count as in the case of dry fruit.

C- Spores bacteria (Flat sour): Boil the water that contains dry vegetables for 5 minutes, then the amount of the evaporated water must be added again as sterile D.W. 2ml of water that contain the Dry vegetables is transferred by pipette to Petri dishes and pour the culture media (Dxtrose/ triptone violet promecrisol agar). Then incubate them for2- 3 days (at 55 ^{o}C). Then, count the colony and estimate the spores' number for each gram.

How to Calculate CFU from Dilution

Colony forming unit (cfu)

cfu=N*D

N=number of colonies, D=dilution

Canned Food

Canning is a method of preserving food in permanent hermetically sealed and sterilized containers (metal, glass, thermo stable plastic or a multilayered flexible pouch). Many cans require opening by cutting the "end" open; others have removable covers. Cans may hold diverse contents such as foods, beverages, oil and chemicals, etc.

Canned foods are sterilized before being placed on the grocery shelf but if the cans most times do occur and usually contain gas produced by members of the genus ***Clostridium***.

Food spoilage is a process in which food deteriorates to the point in which it is not edible to humans, or its quality of edibility becomes reduced. It therefore means that the original nutritional value, texture, flavor of the food is damaged in such a way that the food becomes harmful to people and unsuitable to eat. Furthermore, spoilage may be due to one or more of the following:

- **Physical changes** such as those caused by freezing, burning, drying, and pressure.
- **Chemical reactions** between food and can's material or caused by catalyzing enzymes of microorganisms which occur because the sterilization was not enough to kill them, or the cans were not closed well that allow microorganisms to enter after sterilization.

The important groups of food:

a- Low acid food: Meat, fish, poultry, and dairy fall into a pH range of **5.0-6.8**. This large group is commonly referred to as the low acid group.

b- **Acid food:** With pH range values between **4.5** and **3.7**. Including fruits such as pear, oranges, apricots, and tomatoes fall in this group.

c- **High acid food:** Such as pickled products and fermented foods. The pH values range from **3.7** down to **2.3** also jams and jellies are in this classification.

** Types of Spoiling

A) Spoiling of canned food according to the condition and content of the can:

1- **Swell:** Bulging of both can ends by positive internal pressure due to gas generated by microbial or chemical activity. Either hard or soft swell.

2- **Flipper:** A can with normal appearance, but one end flips out when the can is struck against a solid object, but snaps back to the normal under light pressure.

3- **Springer:** Can bulge from one end which if forced back into normal position, the opposite end bulges.

4- **Leakage:** Due to perforated can or during insufficient sealing process.

5- **Overfilled can:** Has convex ends due to overfilling and not regarded as spoil.

B) Spoiling of canned food according to the cause:

i. **Microbial spoilage**: May result from insufficient sterilization processing or leakage.

ii. **Chemical spoilage**:

a- **Hydrogen swell**: Formation of hydrogen gas in can be due to internal corrosion or scratch. Mainly occurring in acidic food (canned fruits). Quite harmless but undifferentiated from swell of spoiled can, so it is rejected.

b- **Sulphiding** (Sulphur stinker spoilage): Discoloration of can's inside with pink to dark purple. Occur due to reaction of sulphur-containing proteins (liver, kidney, tongue) with liberated H_2S from bacterial spoilage (*Clostridium nigrificans* {Sulphur stinker}) with the odor of rotted eggs. It may be accompanied with blackening when H_2S react with steel base of tin forming iron sulphide and may lead to pitting. Sulphiding can be prevented by sulphur resistant lacquer.

c- **Thermophilic anaerobic spoilage**: *Clostridium thermsccharolyticum* an obligate thermophile causes spoilage. The can swells and may burst due to production of CO_2 and H_2. The food becomes fermented sour, cheesy and develops butyric odor.

d- **Putrefactive anaerobic spoilage**: *Clostridium sporogenes* causes spoilage through putrefaction. The can swells and may burst. Putrefaction result from partial digestion of the food. The latter develops typical putrid odor.

iii. Rust and damage: Rust is reddish brown ferric oxide seen under label.

- Slight rust passes for rapid consumption.
- Sever rust condemned and rejected.
- Damage: Slight damage passes for rapid consumption. Whereas Sever damage rejected.

C) Flat souring: High acid formation without gas production. Sour odor, bitter taste, container not swollen. It caused by thermophilic bacteria:

1- *Bacillus coagulans.*
2- *Bacillus stearothermophilus.*
3- *Bacillus circulans*. These bacteria attack CHO and producing acid without gas.

Microbiological Examination of Eggs

Spoilage of egg is promoted by cracking the eggshell, improper washing, and storage techniques. The most predominate spoilage (rot) of eggs is caused by Gram-negative motile rods: *Pseudomonas*, *Proteus*, *Alcaligenes*, *Aeromonas*, and Coliforms.

Contamination of eggs:

Shells soon become contaminated by:

a. Fecal matter of hen
b. Cage of nest
c. Wash water if eggs are washed
d. Material in which the eggs are packed.

-Changes not caused by M.O.

a. Untreated eggs lose moisture during storage then lose weight
b. Shrinkage shown by the size of the air-cell of the eggs
c. Changes in the physical and chemical states of the egg's contents seen by breaking of the eggs

-Bacterial spoilage of eggs

a. In general, more spoilage of eggs caused by bacteria than by molds.
b. Bacteria need to overcome the anti-bacterial properties of albumin to cause spoilage of eggs.
c. Also, they use the proteins complex as a source of nitrogen and other egg contents as carbon sources for growth.
d. Bacterial spoilage is called as rots. Consist of five types, green, colorless, black, pink and red rots.

Egg rots are:-

1. The green rot: Caused by *Pseudomonas fluorescens* (grows at $0^{o}C$). Egg yolk at early stages disintegrates and blends with white.
2. The colorless rots: Caused by *Pseudomonas*, *Alcaligens* and *Acinetobacter*. Egg yolk in later stages disintegrates or shows a white incrustation.
3. The black rot: Caused by *Pseudomonas*, *Proteus* and *Aeromonas*. These bacteria causes black coloration yolk and dark color in white. This type of spoilage caused when eggs stored at temperature higher than the ordinary.

4. The pink rots: Less often, caused by *Pseudomonas*. Pinkish precipitation on the yolk and a pink color in the egg white.

5. The red rots: Most infrequently occurring one. Caused by a species of Serratia. Some of molds that cause eggs spoilage are species of *Mucor*, *Alternaria* and *Penicillium*.

*The pH of the white may rise from about 7.6 in a freshly laid egg to high as 9.0 to 9.7 within few days (due to loss of CO_2).

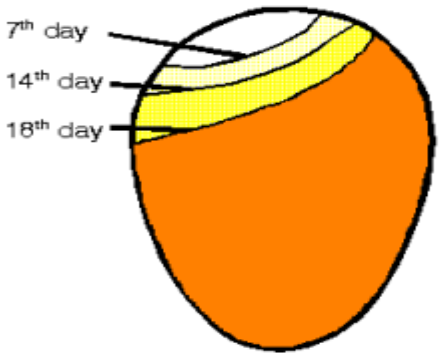
*The M.O. mainly enters the eggs after laying from dirty and moist shell. Washing increase the case due to removal of the bloom and help the microorganisms to invade the egg contents through the pores.

Some examines of eggs:

1- Determine the Shell thickness using a vernia to measure the thickness of different area.
2- Shaking test: Fresh eggs emit no sound, while old eggs emit sound due to thin albumen and free yolk.
3- Air cell size can be used as an indicator of how old the egg is, as air cell is become bigger in the old eggs.

Characteristics of fresh/ high quality egg:

1-yolk is high and firm above the whit.
2-small yolk diameter
3-yolk is centered in the white



Size of air cell on 7th, 14th and 18th day of incubation